FORM PTO-1390 (REV 12-29-99) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: GR1520-BE9512

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 10/009259

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR00/01546	June 6, 2000	June 10, 1999

TITLE OF INVENTION: ELECTROCHEMICAL BIOSENSOR AND CHIP FOR SUCH A BIOSENSOR

APPLICANT(S) FOR DO/EO/US: Jean-Pierre GRASA

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau. (PCT/IB/308).
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:

International Preliminary Examination Report.
Abstract.
Application Data Sheet.
Search Report.

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/009259	PCT/FR00/01546	GR1520-BE9512

17. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):	CALCULATIONS PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1,040.
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	890.
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	740.
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	710.
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	100.
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	29 - 20 =	9	x $ 18.	$ 162
Independent claims	2 - 3 =	0	x 84.	$ 0
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ 280.	$
TOTAL OF ABOVE CALCULATIONS =				$ 1052
Reduction of 1/2 for small entity				$ 526
SUBTOTAL =				$ 526
Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$
TOTAL NATIONAL FEE =				$ 526
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$
TOTAL FEES ENCLOSED =				$ 526
			Amount to be refunded:	$
			charged:	$

a. [X] A check in the amount of $ 526 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required by 37 CFR 1.16 and 1.17, or credit any overpayment to Deposit Account No. 25-0120. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

December 10, 2001

SEND ALL CORRESPONDENCE TO:

Young & Thompson
745 South 23rd Street
2nd Floor
Arlington, VA 22202
(703) 521-2297

CUSTOMER NO. 000466

Benoit Castel
SIGNATURE

Benoit Castel
NAME

35,041
REGISTRATION NUMBER

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Jean-Pierre GRASA

Serial No. (unknown)

Filed herewith

ELECTROCHEMICAL BIOSENSOR AND
CHIP FOR SUCH A BIOSENSOR

PRELIMINARY AMENDMENT

Commissioner for Patents

Washington, D.C. 20231

Sir:

Prior to calculation of the filing fee, please amend the above-identified application as follows:

IN THE CLAIMS:

Amend claim 3 as follows:

--3. (amended) A biosensor as claimed in claim 1, wherein the second electrode (26) is adapted to be directly in contact with the drop (50) of liquid solution.--

Amend claim 4 as follows:

--4. (amended) A biosensor as claimed in claim 1, wherein the second electrode is adapted to be electrically connected to the drop (50) of liquid solution by at least one electrically conductive intermediate element.--

Amend claim 5 as follows:

--5. (amended) A biosensor as claimed in claim 1, wherein the retaining member (26, 70) is formed of the second

electrode (26) directly in contact with the drop (50) of liquid solution.--

Amend claim 6 as follows:

--6. (amended) A biosensor as claimed in claim 1, wherein the retaining member is a specific member (70) distinct from an electrode, and wherein the two electrodes (26, 7) are electrically connected to the reagent chamber (8).--

Amend claim 7 as follows:

--7. (amended) A biosensor as claimed in claim 1, wherein the retaining member (26, 70) has a face (27, 71) facing the contact face (25).--

Amend claim 8 as follows:

--8. (amended) A biosensor as claimed in claim 1, wherein the assembly formed of the retaining member (26, 70) and of the contact face (25) has a face oriented upwards, termed receiving face (25, 65), and wherein the receiving face (25, 65) has an angle of inclination with respect to the horizontal which is greater than 0° and less than 90° - in particular of the order of 40°.--

Amend claim 9 as follows:

--9. (amended) A biosensor as claimed in claim 8, wherein the receiving face is the face (65) of the retaining member (26).--

Amend claim 11 as follows:

--11. (amended) A biosensor as claimed in claim 1, wherein the distance between the retaining member (26, 70) and the contact face (25) is less than 8 mm.--

Amend claim 12 as follows:

--12. (amended) A biosensor as claimed in claim 1, in which the reagent chamber (8) is adapted to enclose a quantity of liquid reactive composition, and has a semi-permeable membrane (9) closing the reagent chamber (8) so as to retain therein the reactive composition, this semi-permeable membrane (9) having a free outer face, forming the said contact face (25), capable of being placed in contact with the liquid solution separated from the reactive composition by the semi-permeable membrane (9),

wherein it has two distinct parts:

- a chip (6) comprising the first electrode (7), the reagent chamber (8) enclosing the liquid reactive composition, and the semi-permeable membrane (9),

- a mount (2) comprising means (11, 12) for receiving a chip (6), means (18, 37, 39) for electrical connection, with an external electrical circuit, of the first electrode (7), of a chip (6) in place in the receiving means (11, 12), this mount (2) carrying the second electrode (26) at a distance from the semi-permeable membrane (9) of a chip (6) in place in the receiving means (11, 12), and comprising means (38, 40)

for electrical connection of the second electrode (26) with the external electrical circuit.--

Amend claim 13 as follows:

--13. (amended) A biosensor as claimed in claim 4, wherein the second electrode (26) comprises a free end (27) extending at a distance from and facing the said receiving face (25) of the semi-permeable membrane (9) of a chip (6), and this free end (27) has an inclined face extending at least substantially parallel to the inclined receiving face (25).--

Amend claim 14 as follows:

--14. (amended) A biosensor as claimed in claim 4, or as claimed in claim 13, wherein the second electrode (26) extends above the receiving face (25) and with a projection downwards with respect to a face (42) of the mount (2) oriented downwards, so that this second electrode (26) has at least one free face (43) extending downwards and oriented upstream with respect to the inclination of the receiving face (25) of the chip (6), and which biosensor comprises a liquid-solution supply shaft (35) emerging immediately upstream of and facing the free face (43) of the second electrode (26), so that the liquid solution is supplied and deposited on this free face (43) to flow downwards along the second electrode (26) until it comes into the gap separating the second electrode (26) and the inclined receiving face (25).--

Amend claim 15 as follows:

--15. A biosensor as claimed in claim 12, wherein the means (11, 12) for receiving a chip comprise an inclined face (11) oriented upwards, and means (12) forming a stop for receiving a chip (6) in the bottom end position on the inclined face (11), wherein the means (18, 37, 39) for electrical connection have an electrical contact stud (18) emerging from the inclined face (11) and adapted to come into electrical connection with a lower conductive portion (17) of the chip (6) in place and in abutment on the inclined face (11), this conductive portion (17) being in electrical connection with the first electrode (7), and wherein the mount (2) comprises a frame (15) carrying the second electrode (26) above, facing and at a distance from the said receiving face (25) of the semi-permeable membrane (9) of a chip (6) in place and in abutment on the inclined face (11).--

Amend claim 17 as follows:

--17. (amended) A biosensor as claimed in claim 12, wherein the mount (2) comprises an orifice (33) for recovery of the liquid solution, arranged so as to be able to recover the liquid solution running off the receiving face (25) and communicating with a lower end (3) of the mount.--

Amend claim 18 as follows:

--18. (Amended) A biosensor as claimed in claim 12, wherein it comprises means (3) for mounting the mount (2) on a receptacle for recovery of the liquid solution.--

Amend claim 21 as follows:

--21. (amended) A chip as claimed in claim 19, wherein the first electrode (7) forms a bottom (19) of the reagent chamber (8) which is closed, opposite this bottom (19), by the semi-permeable membrane (9).--

Amend claim 23 as follows:

--23. (amended) A chip as claimed in claim 21, wherein it has a recessed groove (20) around the bottom (19) formed by the first electrode (7), this groove (20) being adapted to receive a peripheral seal (21) blocking the semi-permeable membrane (9) around and above this bottom (19).--

Amend claim 24 as follows:

--24. (amended) A chip as claimed in claim 19, wherein the first electrode (7) extends so as to have a portion (17) emerging outside the chip (6) in order to form means (17) for electrical connection with an external electrical circuit.--

Amend claim 25 as follows:

--25. (Amended) A chip as claimed in claim 19, wherein it comprises a body (16) of electrically insulating synthetic material in the general shape of a small plate, and wherein the first electrode (7) traverses the thickness of this body (16).--

Amend claim 26 as follows:

--26. (amended) A chip as claimed in claim 19, wherein it is in the general shape of a disc.--

Amend claim 27 as follows:

--27. (amended) A chip as claimed in claim 19, wherein it has a thickness of between 2 mm and 10 mm and a length dimension of between 5 mm and 50 mm.--

Amend claim 28 as follows:

--28. (amended) A chip as claimed in claim 19, wherein the first electrode (7) has a mean radial dimension of between 1 mm and 10 mm - in particular of the order of 4 mm.--

Amend claim 29 as follows:

--29. (amended) A chip as claimed in claim 19, wherein the reactive composition is an enzymatic aqueous solution.--

R E M A R K S

The above changes in the claims merely place this national phase application in the same condition as it was during the international phase, with the multiple dependencies being removed.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE".

Respectfully submitted,

YOUNG & THOMPSON

By

Benoît Castel
Attorney for Applicant
Registration No. 35,041
Customer No. 00466
745 South 23rd Street
Arlington, VA 22202
Telephone: 703/521-2297

December 10, 2001

VERSION WITH MARKINGS TO SHOW CHANGES MADE

The claims have been amended as follows:

3. A biosensor as claimed in ~~one of the preceding claims~~claim 1, wherein the second electrode (26) is adapted to be directly in contact with the drop (50) of liquid solution.

4. A biosensor as claimed in ~~one of claims~~claim ~~1 and~~ 2, wherein the second electrode is adapted to be electrically connected to the drop (50) of liquid solution by at least one electrically conductive intermediate element.

5. A biosensor as claimed in ~~one of the preceding claims~~claim 1, wherein the retaining member (26, 70) is formed of the second electrode (26) directly in contact with the drop (50) of liquid solution.

6. A biosensor as claimed in ~~one of claims~~claim ~~1 to~~ 4, wherein the retaining member is a specific member (70) distinct from an electrode, and wherein the two electrodes (26, 7) are electrically connected to the reagent chamber (8).

7. A biosensor as claimed in ~~one of the preceding claims~~claim 1, wherein the retaining member (26, 70) has a face (27, 71) facing the contact face (25).

8. A biosensor as claimed in ~~one of the preceding claims~~claim 1, wherein the assembly formed of the retaining member (26, 70) and of the contact face (25) has a face oriented upwards, termed receiving face (25, 65), and wherein the receiving face (25, 65) has an angle of inclination with

respect to the horizontal which is greater than 0° and less than 90° - in particular of the order of 40°.

9. A biosensor as claimed in ~~claims 7 and~~claim 8, wherein the receiving face is the face (65) of the retaining member (26).

11. A biosensor as claimed in ~~one of the preceding claims~~claim 1, wherein the distance between the retaining member (26, 70) and the contact face (25) is less than 8 mm.

12. A biosensor as claimed in ~~one of the preceding claims~~claim 1, in which the reagent chamber (8) is adapted to enclose a quantity of liquid reactive composition, and has a semi-permeable membrane (9) closing the reagent chamber (8) so as to retain therein the reactive composition, this semi-permeable membrane (9) having a free outer face, forming the said contact face (25), capable of being placed in contact with the liquid solution separated from the reactive composition by the semi-permeable membrane (9),

wherein it has two distinct parts:

- a chip (6) comprising the first electrode (7), the reagent chamber (8) enclosing the liquid reactive composition, and the semi-permeable membrane (9),

- a mount (2) comprising means (11, 12) for receiving a chip (6), means (18, 37, 39) for electrical connection, with an external electrical circuit, of the first electrode (7), of a chip (6) in place in the receiving means (11, 12), this

mount (2) carrying the second electrode (26) at a distance from the semi-permeable membrane (9) of a chip (6) in place in the receiving means (11, 12), and comprising means (38, 40) for electrical connection of the second electrode (26) with the external electrical circuit.

13. A biosensor as claimed in ~~claims~~claim 4, ~~7, 8, 10 and 12,~~ wherein the second electrode (26) comprises a free end (27) extending at a distance from and facing the said receiving face (25) of the semi-permeable membrane (9) of a chip (6), and this free end (27) has an inclined face extending at least substantially parallel to the inclined receiving face (25).

14. A biosensor as claimed in ~~claims~~claim 4, ~~7, 8, 10 and 12,~~ or as claimed in claim 13, wherein the second electrode (26) extends above the receiving face (25) and with a projection downwards with respect to a face (42) of the mount (2) oriented downwards, so that this second electrode (26) has at least one free face (43) extending downwards and oriented upstream with respect to the inclination of the receiving face (25) of the chip (6), and which biosensor comprises a liquid-solution supply shaft (35) emerging immediately upstream of and facing the free face (43) of the second electrode (26), so that the liquid solution is supplied and deposited on this free face (43) to flow downwards along the second electrode

(26) until it comes into the gap separating the second electrode (26) and the inclined receiving face (25).

15. A biosensor as claimed in ~~one of claims~~claim 12~~to 14~~, wherein the means (11, 12) for receiving a chip comprise an inclined face (11) oriented upwards, and means (12) forming a stop for receiving a chip (6) in the bottom end position on the inclined face (11), wherein the means (18, 37, 39) for electrical connection have an electrical contact stud (18) emerging from the inclined face (11) and adapted to come into electrical connection with a lower conductive portion (17) of the chip (6) in place and in abutment on the inclined face (11), this conductive portion (17) being in electrical connection with the first electrode (7), and wherein the mount (2) comprises a frame (15) carrying the second electrode (26) above, facing and at a distance from the said receiving face (25) of the semi-permeable membrane (9) of a chip (6) in place and in abutment on the inclined face (11).

17. A biosensor as claimed in ~~one of claims~~claim 12~~to 16~~, wherein the mount (2) comprises an orifice (33) for recovery of the liquid solution, arranged so as to be able to recover the liquid solution running off the receiving face (25) and communicating with a lower end (3) of the mount.

18. A biosensor as claimed in ~~one of claims~~claim 12~~to 17~~, wherein it comprises means (3) for mounting the mount (2) on a receptacle for recovery of the liquid solution.

21. A chip as claimed in ~~one of claims~~claim 19~~ and 20~~, wherein the first electrode (7) forms a bottom (19) of the reagent chamber (8) which is closed, opposite this bottom (19), by the semi-permeable membrane (9).

23. A chip as claimed in ~~claims~~claim 21~~ and 22~~, wherein it has a recessed groove (20) around the bottom (19) formed by the first electrode (7), this groove (20) being adapted to receive a peripheral seal (21) blocking the semi-permeable membrane (9) around and above this bottom (19).

24. A chip as claimed in ~~one of claims~~claim 19~~ to 23~~, wherein the first electrode (7) extends so as to have a portion (17) emerging outside the chip (6) in order to form means (17) for electrical connection with an external electrical circuit.

25. A chip as claimed in ~~one of claims~~claim 19~~ to 24~~, wherein it comprises a body (16) of electrically insulating synthetic material in the general shape of a small plate, and wherein the first electrode (7) traverses the thickness of this body (16).

26. A chip as claimed in ~~one of claims~~claim 19~~ to 25~~, wherein it is in the general shape of a disc.

27. A chip as claimed in ~~one of claims~~claim 19~~ to 26~~, wherein it has a thickness of between 2 mm and 10 mm and a length dimension of between 5 mm and 50 mm.

28. A chip as claimed in ~~one of claims~~ claim 19 ~~to 27~~, wherein the first electrode (7) has a mean radial dimension of between 1 mm and 10 mm - in particular of the order of 4 mm.

29. A chip as claimed in ~~one of claims~~ claim 19 ~~to 28~~, wherein the reactive composition is an enzymatic aqueous solution.

ABSTRACT OF THE DISCLOSURE

An electrochemical biosensor includes a first electrode (7) located in a chip (6). The chip (6) can be inserted in a mount (2) having: a second electrode (27) opposite the first electrode (7) and elements (11, 12) for receiving and retaining the chip (6). On the first electrode is deposited a reactive liquid solution maintained and protected by a semi-permeable membrane (9) which is attached to the first electrode (7) by an O-ring seal (21). In measuring mode the chip (6) containing the reactive liquid solution is inserted in the mount (2) and a drop of sample is deposited on the semi-permeable membrane (9). By capillary action, the liquid sample electrically contacts the two electrodes (7, 27) thereby enabling an electrochemical measurement.

ELECTROCHEMICAL BIOSENSOR AND CHIP FOR SUCH A BIOSENSOR

The invention relates to an electrochemical biosensor for measurement of the concentration of a compound in a sample dose of a liquid solution.

The principle and benefits of biosensors have long been known (cf. for example "BIOCAPTEURS: REVE OU REALITE INDUSTRIELLE?" [BIOSENSORS: DREAM OR INDUSTRIAL REALITY?"], Maurice Comtat and Alain Bergel, BIOFUTUR 171, October 1997, p. 33). Nevertheless, their practical applications remain limited in view of the difficulty of implementing them.

In particular, there is a problem with the dosing, the putting into place and renewal of the specific reactive biochemical composition (in particular formed by an enzyme such as glucose oxidase) of the compound whose concentration is to be measured.

In the known apparatuses such as those marketed by INCELTECH FRANCE (Toulouse, France) under the name MICROZYM-L ®, the biosensor comprises a column, the core of which forms a first electrode emerging, outside the column, in a hollow reactant chamber into which a quantity of enzymatic reactive solution is poured by the technician. The chamber is then closed by a semi-permeable membrane covering the end of the column and maintained by an O-ring seal.

A second electrode surrounds the column at a distance from the O-ring seal. The liquid composition is poured onto the membrane, the end of the column being placed upwards. The electrical contact is established between the two electrodes by the reactive solution and the sample liquid solution which flows between the membrane and the second electrode.

These apparatuses require a large volume of liquid solution to be assayed. Some known devices or apparatuses have a reaction cell comprising a well or trough for receiving a dose of liquid solution. In this case, the

wells must be changed at each assay, which is costly and involves time-consuming manipulations.

In addition, in these apparatuses, the changing of the reactive composition is time-consuming (more than 10 minutes of manipulations) and awkward. Moreover, the relatively precise manipulations required are possible in the laboratory but cannot be contemplated in an environment outside laboratories (in industry, agriculture ...).

Equally, these apparatuses do not enable the measurement of the concentration of different compounds, unless they are equipped with a plurality of biosensors, one for each compound to be measured. But in all these cases, the use of a biosensor for each compound is time-consuming and awkward.

Another consequence of this difficulty of putting into place is the fact that the quality of the manipulation can influence the reproducibility and the reliability of the measurements carried out.

One of the solutions envisaged for overcoming these problems consists in immobilising the enzymatic reactive composition on an electrode or on the semi-permeable membrane. Nevertheless, the immobilisation techniques are very difficult to implement. Among these, there may be mentioned: adsorption of the enzyme on a support of the colladion, collagen, cellulose, carbon, silica gel type, etc.; inclusion of the enzyme in a gel or carbon-containing paste or carbon-containing ink; fixation by covalent bonding of the enzyme to a previously activated support.

All these solutions envisaged prove to be complex and costly in terms of fabrication and use.

Furthermore, it should be noted that the considerable cost of the reactive compositions (customarily of the order of (80 FF for a dose of 4µl of glucose oxidase) makes it necessary to be able to keep the same reactive composition as long as it is not degraded. However, the reactive biochemical compositions, especially enzymatic ones, are relatively fragile and unstable.

The object of the invention is therefore to overcome all of these disadvantages by proposing an electrochemical biosensor in which the measurement can be done with a small volume of liquid solvent, in particular a single drop, and the use of which is simple, rapid and inexpensive.

The object of the invention is in particular to propose a biosensor without a reaction cell in the form of a well or trough for receiving the liquid solution. The object of the invention is also to propose a biosensor enabling successive measurements to be carried out rapidly, at lower cost, by minimising the downtimes between two measurements and the unit costs of each measurement.

Furthermore, the object of the invention is to propose a biosensor in which the putting into place of the reactive composition is extremely simple and rapid.

The object of the invention is also to propose a biosensor enabling a rapid change of reactive composition, for example for the measurement of the concentration of different components successively.

The object of the invention is also to propose a biosensor providing reliable results, including in environments outside laboratories (in industry, agriculture ...).

The object of the invention is moreover to propose such a biosensor which is of reduced cost both in terms of fabrication and use.

The object of the invention is thus to propose a biosensor making it possible to satisfy numerous applications in which the known apparatuses cannot be exploited because of the nature or the number of the compounds whose concentration can be measured, and/or the lack of reliability of the results and/or the problems of use and implementation and/or an excessive cost of these known apparatuses.

To do this, the invention relates to an electrochemical biosensor for measurement of the

concentration of a compound in a liquid solution, comprising:

- a reagent chamber adapted to enclose a quantity of biochemical composition, termed reactive composition, and having a free outer face, termed contact face, capable of being placed in contact with the liquid solution which is thus itself in contact with the reactive composition,

- a first electrode and a second electrode which are adapted to be electrically connected to the liquid solution and to enable electrical measurement between them,

wherein it comprises, facing the contact face, a retaining member, the shape of this retaining member and the distance between the contact face and the retaining member being adapted so that a drop of liquid solution placed between the contact face and this retaining member is retained and maintained between them by capillary action. Since the liquid solution to be analysed is formed of a drop retained by capillary action, it is possible to perform assays on solutions available in low volume, a saving on the quantity of liquid solution necessary for the measurement is made, and the manipulations for putting into place and cleaning are extremely simple and rapid. For example, the cleaning can be simply performed manually with the aid of an absorbent or compressed air.

Advantageously and according to the invention, the biosensor is characterized in that the first electrode is electrically connected to the reagent chamber, opposite the contact face, and in that the second electrode is adapted to be electrically connected with the drop of liquid solution on the side of the contact face. The second electrode can be adapted to be directly in contact with the drop of liquid solution, or, in contrast, to be electrically connected to the drop of liquid solution by at least one electrically conductive intermediate element (in particular a film or channel of conductive liquid composition and/or the reagent chamber itself).

In a variant according to the invention, the retaining member is formed of the second electrode directly in contact with the drop of liquid solution, which is in this case interposed between this second electrode and the contact face of the reagent chamber.

In another variant of the invention, the biosensor is characterized in that the retaining member is a specific member distinct from an electrode, and in that the two electrodes are electrically connected (directly or indirectly) to the reagent chamber.

In this way, an electrical connection remains permanently established between the two electrodes via the reagent chamber, thereby avoiding any charge accumulation phenomena (capacitive effects between electrodes), and thus the occurrence of a discharge peak when putting into place a liquid solution to be analysed, adversely affecting the interpretation of the signal.

Be that as it may, advantageously and according to the invention, the retaining member has a face facing the contact face. Thus, the drop is retained between two faces. In a variant, it is possible to provide for the retaining member to be in the shape of a point facing the contact face.

Furthermore, advantageously and according to the invention, the assembly formed of the retaining member and of the contact face has a face oriented upwards, termed receiving face, for the drop of liquid solution. It should be noted nevertheless that since the drop is retained by capillary action, it is also possible to envisage in a variant that the faces or elements (retaining member and contact face) between which it is placed assume any position with respect to the vertical, and in particular horizontally face the other.

Advantageously and according to the invention, the said receiving face has an angle of inclination with respect to the horizontal which is greater than 0° and less than 90° - in particular of the order of 40°. This

inclination is adapted to enable the run-off, owing to gravity, of the excess liquid solution or the drop expelled after the measurement.

In a first variant, the receiving face is the face of the retaining member. In another preferred variant, the receiving face is the contact face of the reactant chamber.

Advantageously and according to the invention, the distance between the retaining member and the contact face is less than 8 mm. Be that as it may, it is known how to determine the distance between two members enabling the insertion and the maintenance by capillary action between them of a drop of solution to be analysed. This distance is associated in particular with the relative values of the surface tensions of the members (retaining member and contact face) and of the liquid solution.

Furthermore, the invention also relates to an electrochemical biosensor for measurement of the concentration of a compound in a dose of a liquid solution, this biosensor comprising:

- a first electrode,

- a reagent chamber adapted to enclose a quantity of liquid biochemical composition, termed reactive composition, and to place it in contact with the first electrode, and having a semi-permeable membrane closing the reagent chamber so as to retain therein the reactive composition, this semi-permeable membrane having a free outer face, forming the said receiving face, capable of being placed in contact with the liquid solution separated from the reactive solution by the semi-permeable membrane,

- a second electrode arranged at a distance from the semi-permeable membrane so as to come into contact with the liquid solution placed against the semi-permeable membrane, wherein it has two distinct parts:

- a chip comprising the first electrode, the reagent chamber enclosing the reactive composition, and the semi-permeable membrane,

- a mount comprising means for receiving a chip, means for electrical connection, with an external electrical circuit, of the first electrode of a chip in place in the receiving means, this mount carrying the second electrode at a distance from the semi-permeable membrane of a chip in place in the receiving means, and comprising means for electrical connection of the second electrode with the external electrical circuit.

Since the reactant chamber pre-dosed with reactive composition and incorporating the first electrode is produced in advance and formed of a part distinct from the mount, which part is denoted throughout the text in a general way by the term "chip", the manipulations and use of the biosensor are greatly facilitated and rapid. The change of reactive composition is virtually instantaneous.

Advantageously and according to the invention, the receiving face of a chip in place in the receiving means is oriented upwards and has a non-zero angle of inclination with respect to the horizontal. This inclination is adapted to enable the run-off, owing to gravity, of the excess liquid solution downwards, without coming directly into contact with the first electrode or its means of electrical connection arranged below the chip. Advantageously and according to the invention, the said angle of inclination is between 0° and 90° - in particular of the order of 40°. This inclination can be obtained by an inclination of a face of the mount receiving the chip and/or by a specific shape given to the chip (for example the shape of a wedge).

Advantageously and according to the invention, the second electrode comprises a free end extending at a distance from and facing the said receiving face of the semi-permeable membrane of a chip, and this free end has an inclined face extending at least substantially parallel to the inclined receiving face. Advantageously and according to the invention, the second electrode has a free end extending at a distance from the said receiving face of the semi-permeable membrane of the chip, which is adapted to

retain the drop of liquid solution by capillary action - in particular less than 8 mm.

In addition, advantageously and according to the invention, the biosensor is characterized in that the second electrode extends above the receiving face and with a projection downwards with respect to a face of the mount oriented downwards, so that this second electrode has at least one free face extending downwards and oriented upstream with respect to the inclination of the receiving face of the chip, and in that it comprises a liquid-solution supply shaft emerging immediately upstream of and facing the free face of the second electrode, so that the liquid solution is supplied and deposited on this free face to flow downwards along the second electrode until it comes into the gap separating the second electrode and the inclined receiving face.

Advantageously and according to the invention, the biosensor is also characterized in that the means for receiving a chip comprise an inclined face oriented upwards (so as to support a chip placed on this inclined face), and means forming a stop for receiving a chip in the bottom end position on the inclined face, in that the means for electrical connection have an electrical contact stud emerging from the inclined face and adapted to come into electrical connection with a lower conductive portion of the chip in place and in abutment on the inclined face, this conductive portion itself being in electrical connection with the first electrode, and in that the mount comprises a frame carrying the second electrode above, facing and at a distance from the said receiving face of the semi-permeable membrane of a chip in place and in abutment on the inclined face. Advantageously and according to the invention, the biosensor comprises means for pressing the chip against the inclined face. Advantageously and according to the invention, the mount comprises an orifice for recovery of the liquid solution, arranged so as to be able to recover the liquid solution running off the

receiving face - in particular made in the lower part of the inclined face - after the measurement and communicating with a lower end of the mount. Advantageously and according to the invention, the biosensor comprises means for mounting the mount on a receptacle for recovery of the liquid solution.

The object of the invention is also to propose a chip for a biosensor according to the invention. To this end, the object of the invention is to propose a package, which is reliable, of low cost and easy to manipulate, for a dose of reactive liquid biochemical composition - in particular an enzymatic solution - intended for a biosensor.

To do this, the invention extends to an electrochemical biosensor chip for measurement of the concentration of a compound in a liquid solution, wherein it comprises:

- a reagent chamber enclosing a quantity of liquid biochemical composition, termed reactive composition, and having a semi-permeable membrane closing the reagent chamber so as to retain therein the reactive composition, this semi-permeable membrane having a free outer face, termed receiving face, capable of receiving a drop of liquid solution separated from the reactive composition by the semi-permeable membrane,

- an electrode, termed first electrode, placed in electrical contact with the reactive composition contained in the reagent chamber, and means for electrical connection of this first electrode with an electrical circuit outside the chip.

The invention also relates to a chip adapted to be used with a biosensor according to the invention. The chip according to the invention is simultaneously a consumable package of reactive composition and a support for the first electrode, which can equally well be an anode or a cathode, the second electrode then being a cathode or, respectively, an anode.

Advantageously and according to the invention, the first electrode has an end emerging in the reagent chamber opposite a portion of the semi-permeable membrane forming the said receiving face. Advantageously and according to the invention, the first electrode forms a bottom of the reagent chamber which is closed, opposite this bottom, by the semi-permeable membrane. Advantageously and according to the invention, the reagent chamber is delimited by the bottom formed by the first electrode and by the semi-permeable membrane extending from the bottom and above the bottom. Advantageously and according to the invention, the chip has a recessed groove around the bottom formed by the first electrode, this groove being adapted to receive a peripheral seal blocking the semi-permeable membrane around and above this bottom. Advantageously and according to the invention, the first electrode extends so as to have a portion emerging outside the chip in order to form means for electrical connection with an external electrical circuit. Advantageously and according to the invention, the chip comprises a body of electrically insulating synthetic material in the general shape of a small plate, and the first electrode traverses the thickness of this body. In a variant, the first electrode can be connected to a conducting wire itself emerging outside the chip and adapted to be able to be connected to an external electrical circuit.

Advantageously and according to the invention, the chip is in the general shape of a disc. A chip according to the invention advantageously has a thickness of between 2 mm and 10 mm - in particular of the order of 4 mm - and a length dimension (or diameter) of between 5 mm and 50 mm - in particular of the order of 20 mm. Advantageously and according to the invention, the first electrode has a mean radial dimension of between 1 mm and 10 mm - in particular of the order of 4 mm.

More particularly and advantageously, in a biosensor and a chip according to the invention, the reactive

composition is an enzymatic aqueous solution, and the electrodes are of the amperometric detection type (anode and cathode). When the reactive composition is an enzymatic solution incorporating a dehydrogenase enzyme with a couple associated with the Fe^{++}/Fe^{+++} couple, the first electrode is an anode. Nevertheless, the invention also extends to any other reactive biochemical composition, liquid or non-liquid, compatible with a biosensor of the electrochemical type having two electrodes.

The invention also relates to a biosensor and a chip characterized in combination by all or part of the features mentioned hereinabove or hereinbelow.

The invention enables easy manipulation of the liquid solution to be assayed and of the package (chip) of the reactive composition and greatly facilitates the use and renewal of the doses of liquid solution to be assayed and of reactive composition, which are instantaneous operations. Moreover, the reactive composition is protected from the outside environment and can be preserved, stored and re-used according to the compound whose concentration is being measured. The invention can also be implemented and used in any environments whatsoever, without special precautions (industry, agriculture, sports ground or outdoors ...), in a simple way and makes it possible to obtain reliable results. In addition, it is very simple and inexpensive to fabricate.

Other objects, features and advantages of the invention will become apparent on reading the following description which refers to the appended figures in which:

- Figure 1 is a schematic perspective view of a biosensor according to a first preferred embodiment of the invention,

- Figure 2 is a schematic view, in section on a median axial vertical plane, of the biosensor of Figure 1,

- Figure 3 is a schematic view, from the left, of the biosensor of Figure 2,

- Figure 4 is a schematic view in section on the line IV-IV of Figure 2,

- Figure 5 is a schematic perspective view of a chip according to a first embodiment of the invention,

- Figures 6a, 6b, 6c illustrate schematically, in section on a median axial vertical plane, three successive stages of the fabrication of the chip of Figure 5,

- Figure 7 is a schematic view, in section on a median axial vertical plane, of a chip according to a second embodiment of the invention,

- Figure 8 is a schematic view, in exploded section on a median axial vertical plane, illustrating the constituent elements of a chip according to the second embodiment of the invention,

- Figures 9, 10 and 11 are partial diagrams, in section on a median axial vertical plane, illustrating three other embodiments of a biosensor according to the invention.

The electrochemical biosensor 1 according to the invention shown in Figures 1 to 4 comprises a mount 2 made of electrically insulating synthetic material with the general shape overall of a cylindrical solid of revolution of vertical axis, intended to be mounted by its lower tapped end 3 on an upper threaded end 4 of a receptacle such as a bottle intended to collect sample doses of liquid solution after analysis in the biosensor 1.

The biosensor 1 comprises an analysis zone 5 into which the doses of liquid solution to be analysed can be introduced. The analysis zone 5 incorporates the various devices enabling the measurements of the concentration in the liquid solution to performed and defines a compartment for receiving a chip 6 formed of a part distinct from the mount 2, and removable from the mount 2.

This chip 6 comprises a first electrode 7, a reagent chamber 8 enclosing a predetermined quantity of liquid biochemical composition, termed reactive composition, such as an enzymatic solution, this reagent chamber 8 being

closed by a semi-permeable membrane 9 so that the reactive composition is retained within the reagent chamber 8 and remains in contact with the first electrode 7.

The compartment for receiving the chip 6 comprises a lateral introduction slot 10 made in the mount 2, an inclined face 11 oriented upwards and extending from the slot 10 radially downwards at a non-zero angle of inclination greater than 0° and less than or equal to 90° - in particular of the order of 40° - with respect to the horizontal and, opposite the introduction slot 10, means 12 forming a stop for receiving the chip 6 in the bottom end position on the inclined face 11. In the embodiment shown, these means 12 forming a stop consist of two lateral uprights 13 extending the inclined face 11 upwards on each side and forming, with an upper end head 14 of the mount 2, a frame 15 which covers the inclined face 11 closing the compartment for receiving the chip 6 and the analysis zone 5. As can be seen in Figure 4, the two lateral uprights 13 have a shape adapted to receive between them a chip 6 placed on the inclined face 11 while blocking it in abutment in the lower end position on the inclined face 11.

The chip 6 comprises a body 16 made of electrically insulating synthetic material, in the general shape of a small plate and of a section of a cylindrical solid of revolution of low thickness, that is to say in the general shape of a disc. This body 16 is traversed in its thickness axially by the first electrode 7 which is formed of an electrically conductive but electrolytically resistant material. The lower end 17 of the first electrode 7 emerges outside the lower face of the body 16 of the chip 6, and extends with a slight projection with respect to this lower face, so as to come into electrical contact with an electrical contact stud 18 emerging from the inclined face 11, when the chip 6 is in place, in abutment 12 against the lateral uprights 13 on the inclined face 11. The upper end 19 of the first electrode 7 forms the bottom of the reagent chamber 8 of the chip 6. The reactive composition contained

in this reagent chamber 8 is thus in contact with the first electrode 7. The semi-permeable membrane 9 retains the reactive composition above the upper end 19 of the first electrode 7, which it covers. In these figures, the relative scales in terms of thickness are not adhered to, for the purposes of illustration. Thus, the reagent chamber 8 is shown much thicker with respect to the thickness of the body 16 than it is in reality.

A first embodiment of a chip 6 according to the invention is shown in Figures 2, 3, 4, 5, 6a to 6c. In this first embodiment, a groove 20 is recessed in the body 16 all around the bottom 19 formed by the first electrode 7, so that this peripheral groove 20 is able to receive a peripheral seal 21 blocking the semi-permeable membrane 9 around and above the bottom 19. In this first embodiment, the reagent chamber 8 is thus entirely delimited on the one hand by the upper end of the first electrode 7 forming the bottom 19, and on the other hand by the semi-permeable membrane 9 which retains the reactive composition against this bottom 19 and forms a pocket containing it, being blocked by the peripheral seal 21 placed in the groove 20.

As can be seen in Figures 6a to 6c, to fabricate such a chip 6, starting with the body 16 in which the first electrode 7 has been engaged, a predetermined quantity in the form of a drop of a reactive composition 22 is deposited (Figure 6b) on the upper end 19 of the first electrode 7, then the semi-permeable membrane 9 is placed on top of this drop 22, blocking it with respect to the first electrode 7 by virtue of the seal 21 engaged in the groove 20 (Figure 6c).

Figures 7 and 8 show a second embodiment of the chip 6. In this embodiment, the upper face 41 of the body 16 of the chip 6 is plane and flush with the upper end 19 of the first electrode 17. A double-faced adhesive sheet 23, pierced at its centre with a circular perforation 24 with a diameter less than or equal to that of the upper end 19 of the first electrode 7, is bonded to the body 16. The

perforation 24 is arranged above the upper end 19 of the first electrode 7 and is centred on this upper end 19. The semi-permeable membrane 9 is in the form of a sheet, itself bonded on top of the double-faced adhesive sheet 23, so that the semi-permeable membrane 9 closes the perforation 24 which thus defines the volume of the reagent chamber 8 in which the reactive composition is placed. To fabricate this chip, an adhesive sheet 23 is bonded on top of the semi-permeable membrane 9, then a drop 22 of reactive composition is deposited in the perforation 24, then the body 16 equipped with the first electrode 7 is presented, inverted with the end 19 and the face 41 oriented downwards, to be bonded to the adhesive sheet 23, thereby closing the perforation 24. Subsequently, all that is required is to invert the assembly thus formed to obtain the chip 6 of Figure 7. In place of a self-adhesive sheet 23, it is also possible to use a sheet of synthetic material of predetermined thickness bonded to the semi-permeable membrane 9 and to the body 16 by a suitable adhesive, for example of the cyanocrylate type.

In the two embodiments shown, the chip 6 has an at least substantially plane, free outer face, termed receiving face 25, extending overall parallel to the bottom 19 formed by the upper end of the first electrode 7, and to the body 16, and this receiving face 25 is adapted to receive a drop 50 of liquid solution to be analysed, this drop of liquid solution being separated from the reactive composition enclosed in the reagent chamber 8 by the semi-permeable membrane 9. The receiving face 25 is thus also a contact face, that is to say it comes into contact with the liquid solution to be analysed. Nevertheless, ionic and electronic exchanges through the semi-permeable membrane 9 are possible, so that an oxidoreduction reaction occurs, depending on the concentration of the compound corresponding to the enzymatic biochemical agent contained in the reagent composition.

The frame 15 of the biosensor also carries a second electrode 26 arranged at a distance from the semi-permeable membrane 9 so as to come into contact with the drop of liquid solution which is placed on the receiving face 25 of the semi-permeable membrane 9, as shown in Figure 2. The second electrode 26 is arranged above, facing and at a distance from the receiving face 25 of a chip 6 in place on the inclined face 11, and this second electrode 26 has a lower free end 27 comprising an inclined plane face extending at least substantially parallel to the receiving face 25 which is itself inclined at least substantially parallel to the inclined face 11, that is to say at an angle of between 0° and 90°, preferably between 10° and 75° - in particular of the order of 40° - with respect to the horizontal. The distance provided between the lower free end 27 of the second electrode 26 and the receiving face 25 of the semi-permeable membrane 9 of the chip 6 is adapted so that a drop 50 of liquid solution to be analysed is retained by capillary action in the space provided between this free end 27 and this receiving face 25. This distance thus depends in particular on the diameter of the second electrode 26 and of the receiving face 25 which is preferably between 1 mm and 10 mm - in particular of the order of 4 mm -, on the surface tensions of the receiving face 25 and of the free face 27 of the second electrode 26, on the viscosity (hence the surface tension) of the liquid solution to be analysed, and on the inclination of the receiving face 25. In practice, advantageously and according to the invention, this distance is less than 8 mm and greater than 1 mm.

A chip 6 according to the invention typically has a thickness of between 2 mm and 10 mm - in particular of the order of 4 mm -, the thickness of the reagent chamber 8 being less than 1 mm, of the order of a few microns to a few tens of millimetres. The chip 6 advantageously has a length dimension (greatest dimension perpendicularly to the thickness, that is to say radial dimension, or diameter if

it is in the shape of a disc) of between 5 mm and 50 mm - in particular of the order of 20 mm. The free end face 27 of the second electrode 26 and the receiving face 25 have a mean dimension parallel to the plane of the receiving face 25 (in particular a diameter in the embodiments in which these faces are circular) of between 1 mm and 10 mm - in particular of the order of 5 mm. The electrodes 7, 26 have a mean radial dimension (diameter if they are cylindrical solids of revolution) of between 1 mm and 10 mm - in particular of the order of 4 mm.

The first electrode 7 is preferably in the shape of a cylindrical solid of revolution, the receiving face 25 being in the general shape of a disc. Similarly, the second electrode 26 is preferably in the shape of a cylindrical solid of revolution. Nevertheless, there is nothing to prevent, in variants not shown, other forms of embodiment (for example prismatic electrodes 7, 26 and polygonal receiving face 25 ...) being provided. Similarly, the body 16 of the chip 6 may not be cylindrical, but prismatic, or even in the shape of a wedge (with two bases not parallel to each other) so as to form or participate in the inclination of the receiving face 25 with respect to the horizontal (the inclined face 11 being less inclined or even not inclined).

Furthermore, the biosensor 1 comprises a metal clamp 28 comprising two vertical limbs 29 engaged in corresponding vertical bores made through the head 14 of the frame 15 so that the lower ends 30 of these limbs 29 come to bear against the body 16 of a chip 6 in place on the inclined face 11. The clamp 28 thus presses, owing to the effect of its weight, the chip 6 against the inclined face 11, and the first electrode 7 into electrical contact with the stud 18. The clamp 28 comprises an upper crosspiece 31 connecting the two vertical limbs 29 and equipped with a vertical manipulating extension 32 enabling the user to raise the clamp 28 in order to free the chip 6. In a variant not shown, the clamp 28 can also be returned

into contact with the chip 6 by spring return means. The lower free ends 30 of the limbs 29 of the clamp 28 are preferably bevelled so as to extend parallel to the inclined plane 11 and the body 16.

The biosensor 1 further comprises an orifice 33 for recovery of the liquid solution after analysis, and this orifice 33 is made in the lower part of the inclined face 11, so that the liquid solution runs off into the orifice 33 naturally owing to gravity from the receiving face 25, without coming into contact with the first electrode 7 or the contact stud 18. This run-off can be brought about by the operator expelling the drop (maintained by capillary action) downwards, mechanically or with a compressed air jet. An absorbent element (cotton wool, cloth ...) can also be used. The orifice 33 communicates via a vertical conduit 34 with the lower end 3 of the mount 2, so that the liquid composition can run off into the recovery receptacle on which the biosensor 1 is mounted.

Furthermore, the frame 15 of the biosensor 1 comprises a liquid-solution supply shaft 35, through which the end of a pipette 36 can be introduced to enable the delivery of a dose of liquid solution to be analysed corresponding to the drop 50. This supply shaft 35 emerges immediately upstream of and facing a vertical free face 43 of the second electrode 26, this free face 43 being oriented upstream with respect to the inclination of the receiving face 25 of the chip 6, and thus with respect to the direction of flow of the liquid solution over this receiving face 25.

The second electrode 26 extends above and facing the receiving face 25. It is carried by the frame 15 of the mount 2 which has a free lower face 42 oriented downwards, extending above and at a distance from the inclined face 11 in order to form the compartment for receiving the chip 6. The second electrode 26 extends with a projection downwards from this lower face 42 of the frame 15 so as to present the said free face 43, against which the end 44 of a pipette 36 engaged in the supply shaft 35 can be placed in

order to deposit there a dose (drop 50) of liquid solution. The drop 50 of liquid solution thus deposited flows downwards owing to gravity to fill the space between the second electrode 26 and the receiving face 25, and it is maintained there by capillary action. If an excess of liquid solution is deposited, the latter will run off owing to gravity downwards over the inclined chip 6, then into the recovery orifice 33, by virtue of the inclination of the receiving face 25 and of the chip 6 which is adapted for this purpose.

The contact stud 18 and the second electrode 26 are connected to an external electrical circuit by a conducting wire 37 and 38, respectively, which emerge outside the mount 2. Each of these wires 37, 38 is passed through a conduit made through the mount 2 to emerge outside the mount 2. Each wire 37, 38 has, at its outer free end, a connector 39 and 40, respectively, for its connection to an external electrical circuit to which is supplied the electric current corresponding to the measurement of the concentration of the chemical compound in the liquid solution to be analysed, in particular for the measurement of the intensity of the electric current in the case of electrodes 7, 26 of the amperometric type.

The first electrode 7 carried by a chip 6 is connected to the wire 37 via the contact stud 18 to which the wire 37 is welded. In contrast, the wire 38 of the second electrode 26 is directly welded to this second electrode 26.

The semi-permeable membrane 9 used in a chip 6 of the invention can be a semi-permeable membrane made of cellophane such as a dialysis membrane. The electrodes 7, 26 can be made entirely of gold or platinum, or of gold- or platinum-plated metal alloy, or of any other electrically conductive and electrolytically resistant material.

The chip 6 is immediately removable from the mount 2 by simple manual manipulation without tools. To remove a chip 6 in place in the analysis zone 5, all that is required in fact is to raise the clamp 28 by pulling the

manipulating extension 32 upwards, and to extract the chip 6 through the slot 10 by sliding it upwards on the inclined face 11. To put a new chip 6 in place, it is introduced into the slot 10 until it comes into lower abutment 12 against the lateral uprights 13, then the clamp 28 is released and its limbs 29 come into contact with the body 16 of the chip 6, pressing it onto the inclined face 11. The chip 6 is thus automatically precisely positioned with respect to the contact stud 18 and with respect to the second electrode 26. A drop of a liquid solution to be analysed can immediately be injected with a pipette 36 as shown in Figure 2.

This embodiment of the invention is particularly applicable advantageously to the production of a biosensor usable wherever it is necessary to confine a reactive biochemical liquid composition in a reagent chamber 8 separated from a liquid solution to be analysed, by a semi-permeable membrane 9, one electrode being in contact with the liquid composition to be analysed, whereas the other electrode is in contact with the reactive solution. The invention is especially applicable to the measurement of the concentration of a chemical compound in an aqueous liquid solution of natural or artificial origin, with an enzymatic reactive composition. The value of the concentration is obtained by measuring the current flowing between the electrodes following the oxidoreduction enzymatic chemical reaction generated in the liquid solution by the enzymatic reactive composition. It is thus possible, in particular, to measure the glucose or lactate concentrations precisely and rapidly.

The invention nevertheless applies equally well to the measurement of any other chemical compound, and in particular to the detection and measurement of concentrations of various compounds in wines, agri-food substances, and physiological fluids. Thus, the invention can have a great number of applications: determination of glucose, lactate, urea, cholesterol, or other metabolites

(hormones, antibodies ...), alcohols or illicit drugs, by taking a sample of a liquid, blood or other solution from the human or animal organism; determination of compounds such as sugars, amino acids, glutamate, lactate, ... in the finished products or preparation processes of agri-food products such as bread, milk, dairy products, wines, beers ...; measurement of the ripeness of fruits; measurement of the freshness of fish and meats; determination of the bacterial contamination of an agri-food product; determination of toxic agents in industrial or natural liquid solutions (pesticides, fungicides, nitrates, phenols, organochlorine or organophosphorous compounds, metallic binders; measurement of the biological oxygen demand, detection of organic contamination of waters ...)...

Furthermore, the invention can have numerous variants from the embodiments described and shown in Figures 1 to 8. In particular, the form, dimensions, and material from which the biosensor 1 and the chips 6 are made can vary widely. The mount 2 can itself be made of a single moulded and/or machined part, or preferably of a plurality of moulded and/or machined parts assembled subsequently.

As can be seen in Figure 9, the chip can be formed of a solid or semi-solid chip 60 of reactive composition immobilised in the form of a gel, formed entirely of the reagent chamber 8, and which can be removably mounted in a housing of the mount 2.

The variant of Figure 10 shows that the system can also be inverted, with the reagent chamber formed of the chip 60 and the first electrode 7 placed above, and the second electrode 26 being placed below, acting as the member for retaining the drop 50, and having a face 65 for receiving the drop 50.

The system can also be placed in any other position.

Furthermore, as can be seen in Figure 11, the drop 50 can be maintained between the reagent chamber 8 and a part 70 distinct from the second electrode 26 (and also of

course from the first electrode 7). This part 70 has a face 71 facing the free contact face 25 of the reagent chamber 8. The second electrode 26 can in this case be adapted to come into contact with a channel 72 filled with water or conducting solution and recessed in the chip 6 towards the outside radially from the membrane 9. This channel 72 can be sufficiently fine to retain the water or conducting solution by capillary action. In a variant not shown, it can be placed on the side so as to extend laterally horizontally even when the free face 25 of the reagent chamber 8 and that 71 of the part 70 are inclined.

These different variants can be combined.

CLAIMS

1. An electrochemical biosensor for measurement of the concentration of a compound in a liquid solution, comprising:

- a reagent chamber (8) adapted to enclose a quantity of biochemical composition, termed reactive composition, and having a free outer face, termed contact face (25), capable of being placed in contact with the liquid solution which is thus itself in contact with the reactive composition,

- a first electrode (7) and a second electrode (26) which are adapted to be electrically connected to the liquid solution and to enable electrical measurement between them,

wherein it comprises, facing the contact face (25), a retaining member (26, 70), the shape of this retaining member (26, 70) and the distance between the contact face (25) and the retaining member (26, 70) being adapted so that a drop (50) of liquid solution placed between the contact face (25) and this retaining member (26, 70) is retained and maintained between them by capillary action.

2. A biosensor as claimed in claim 1, wherein the first electrode (7) is electrically connected to the reagent chamber (8), opposite the contact face (25), and wherein the second electrode (26) is adapted to be electrically connected with the drop (50) of liquid solution on the side of the contact face (25).

3. A biosensor as claimed in one of the preceding claims, wherein the second electrode (26) is adapted to be directly in contact with the drop (50) of liquid solution.

4. A biosensor as claimed in one of claims 1 and 2, wherein the second electrode is adapted to be electrically connected to the drop (50) of liquid solution by at least one electrically conductive intermediate element.

5. A biosensor as claimed in one of the preceding claims, wherein the retaining member (26, 70) is formed of

the second electrode (26) directly in contact with the drop (50) of liquid solution.

6. A biosensor as claimed in one of claims 1 to 4, wherein the retaining member is a specific member (70) distinct from an electrode, and wherein the two electrodes (26, 7) are electrically connected to the reagent chamber (8).

7. A biosensor as claimed in one of the preceding claims, wherein the retaining member (26, 70) has a face (27, 71) facing the contact face (25).

8. A biosensor as claimed in one of the preceding claims, wherein the assembly formed of the retaining member (26, 70) and of the contact face (25) has a face oriented upwards, termed receiving face (25, 65), and wherein the receiving face (25, 65) has an angle of inclination with respect to the horizontal which is greater than 0° and less than 90° - in particular of the order of 40°.

9. A biosensor as claimed in claims 7 and 8, wherein the receiving face is the face (65) of the retaining member (26).

10. A biosensor as claimed in claim 8, wherein the receiving face is the contact face (25) of the reagent chamber (8).

11. A biosensor as claimed in one of the preceding claims, wherein the distance between the retaining member (26, 70) and the contact face (25) is less than 8 mm.

12. A biosensor as claimed in one of the preceding claims, in which the reagent chamber (8) is adapted to enclose a quantity of liquid reactive composition, and has a semi-permeable membrane (9) closing the reagent chamber (8) so as to retain therein the reactive composition, this semi-permeable membrane (9) having a free outer face, forming the said contact face (25), capable of being placed in contact with the liquid solution separated from the reactive composition by the semi-permeable membrane (9), wherein it has two distinct parts:

- a chip (6) comprising the first electrode (7), the reagent chamber (8) enclosing the liquid reactive composition, and the semi-permeable membrane (9),

- a mount (2) comprising means (11, 12) for receiving a chip (6), means (18, 37, 39) for electrical connection, with an external electrical circuit, of the first electrode (7), of a chip (6) in place in the receiving means (11, 12), this mount (2) carrying the second electrode (26) at a distance from the semi-permeable membrane (9) of a chip (6) in place in the receiving means (11, 12), and comprising means (38, 40) for electrical connection of the second electrode (26) with the external electrical circuit.

13. A biosensor as claimed in claims 4, 7, 8, 10 and 12, wherein the second electrode (26) comprises a free end (27) extending at a distance from and facing the said receiving face (25) of the semi-permeable membrane (9) of a chip (6), and this free end (27) has an inclined face extending at least substantially parallel to the inclined receiving face (25).

14. A biosensor as claimed in claims 4, 7, 8, 10 and 12, or as claimed in claim 13, wherein the second electrode (26) extends above the receiving face (25) and with a projection downwards with respect to a face (42) of the mount (2) oriented downwards, so that this second electrode (26) has at least one free face (43) extending downwards and oriented upstream with respect to the inclination of the receiving face (25) of the chip (6), and which biosensor comprises a liquid-solution supply shaft (35) emerging immediately upstream of and facing the free face (43) of the second electrode (26), so that the liquid solution is supplied and deposited on this free face (43) to flow downwards along the second electrode (26) until it comes into the gap separating the second electrode (26) and the inclined receiving face (25).

15. A biosensor as claimed in one of claims 12 to 14, wherein the means (11, 12) for receiving a chip comprise an inclined face (11) oriented upwards, and means (12) forming

a stop for receiving a chip (6) in the bottom end position on the inclined face (11), wherein the means (18, 37, 39) for electrical connection have an electrical contact stud (18) emerging from the inclined face (11) and adapted to come into electrical connection with a lower conductive portion (17) of the chip (6) in place and in abutment on the inclined face (11), this conductive portion (17) being in electrical connection with the first electrode (7), and wherein the mount (2) comprises a frame (15) carrying the second electrode (26) above, facing and at a distance from the said receiving face (25) of the semi-permeable membrane (9) of a chip (6) in place and in abutment on the inclined face (11).

16. A biosensor as claimed in claim 15, wherein it comprises means (28) for pressing the chip (6) against the inclined face (11).

17. A biosensor as claimed in one of claims 12 to 16, wherein the mount (2) comprises an orifice (33) for recovery of the liquid solution, arranged so as to be able to recover the liquid solution running off the receiving face (25) and communicating with a lower end (3) of the mount.

18. A biosensor as claimed in one of claims 12 to 17, wherein it comprises means (3) for mounting the mount (2) on a receptacle for recovery of the liquid solution.

19. An electrochemical biosensor chip for measurement of the concentration of a compound in a liquid solution, wherein it comprises:

- a reagent chamber (8) enclosing a quantity of liquid biochemical composition, termed reactive composition, and having a semi-permeable membrane (9) closing the reagent chamber (8) so as to retain therein the reactive composition, this semi-permeable membrane (9) having a free outer face, termed receiving face (25), capable of receiving a drop of liquid solution separated from the reactive composition by the semi-permeable membrane (9),

- an electrode, termed first electrode (7), placed in electrical contact with the reactive composition contained in the reagent chamber (8), and means (17) for electrical connection of this first electrode (7) with an electrical circuit outside the chip (6).

20. A chip as claimed in claim 19, wherein the first electrode (7) has an end (19) emerging in the reagent chamber (8) opposite a portion of the semi-permeable membrane (9) forming the said receiving face (25).

21. A chip as claimed in one of claims 19 and 20, wherein the first electrode (7) forms a bottom (19) of the reagent chamber (8) which is closed, opposite this bottom (19), by the semi-permeable membrane (9).

22. A chip as claimed in claim 21, wherein the reagent chamber (8) is delimited by the bottom (19) formed by the first electrode (7) and by the semi-permeable membrane (9) extending from the bottom (19) and above the bottom (19).

23. A chip as claimed in claims 21 and 22, wherein it has a recessed groove (20) around the bottom (19) formed by the first electrode (7), this groove (20) being adapted to receive a peripheral seal (21) blocking the semi-permeable membrane (9) around and above this bottom (19).

24. A chip as claimed in one of claims 19 to 23, wherein the first electrode (7) extends so as to have a portion (17) emerging outside the chip (6) in order to form means (17) for electrical connection with an external electrical circuit.

25. A chip as claimed in one of claims 19 to 24, wherein it comprises a body (16) of electrically insulating synthetic material in the general shape of a small plate, and wherein the first electrode (7) traverses the thickness of this body (16).

26. A chip as claimed in one of claims 19 to 25, wherein it is in the general shape of a disc.

27. A chip as claimed in one of claims 19 to 26, wherein it has a thickness of between 2 mm and 10 mm and a length dimension of between 5 mm and 50 mm.

28. A chip as claimed in one of claims 19 to 27, wherein the first electrode (7) has a mean radial dimension of between 1 mm and 10 mm - in particular of the order of 4 mm.

29. A chip as claimed in one of claims 19 to 28, wherein the reactive composition is an enzymatic aqueous solution.

Fig 1

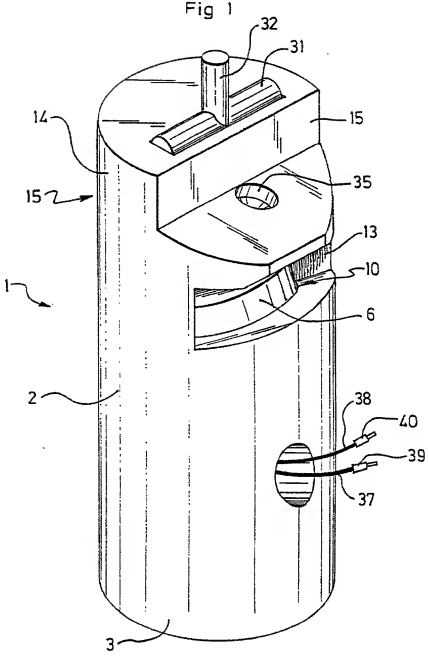

Fig 2

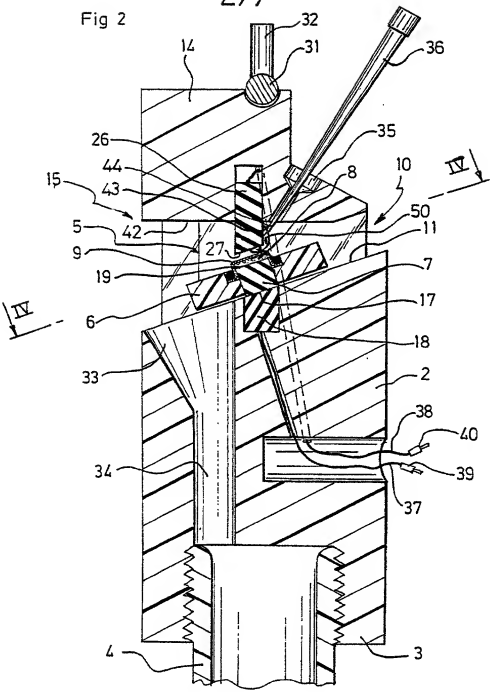
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Fig 3

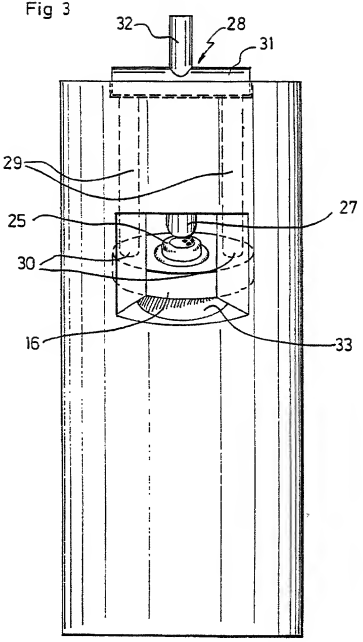


Fig 4

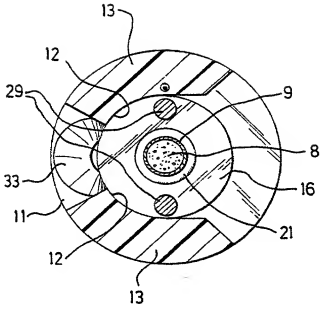


Fig 5

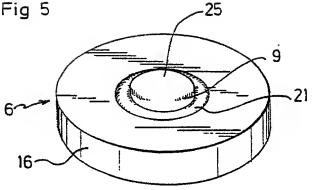


Fig 6a

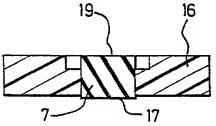


Fig 6b

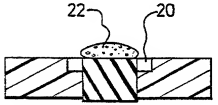


Fig 6c

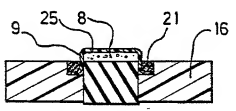


Fig 7

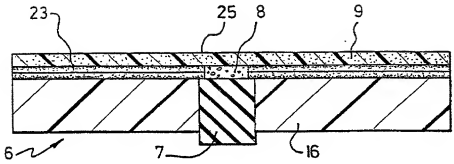


Fig 8

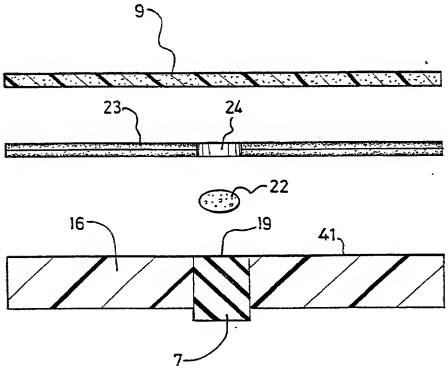


Fig 9

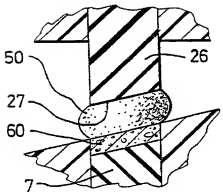


Fig 10

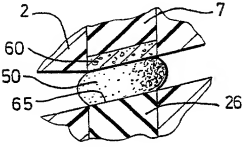
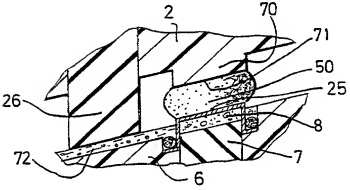


Fig 11



Declaration and Power of Attorney for Patent Application

Déclaration et Pouvoirs pour Demande de Brevet

French Language Declaration

En tant que l'inventeur nommé ci-après, je déclare par le présent acte que:

As a below named inventor, I hereby declare that:

Mon domicile, mon adresse postale et ma nationalité sont ceux figurant ci-dessous à côté de mon nom.

My residence, post office address and citizenship are as stated next to my name.

Je crois être le premier inventeur original et unique (si un seul nom est mentionné ci-dessous), ou l'un des premiers co-inventeurs originaux (si plusieurs noms sont mentionnés ci-dessous) de l'objet revendiqué, pour lequel une demande de brevet a été déposée concernant l'invention intitulée

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

BIOCAPTEUR ELECTROCHIMIQUE ET PASTILLE POUR UN TEL BIOCAPTEUR

et dont la description est fournie ci-joint à moins que la case suivante n'ait été cochée:

the specification of which is attached hereto unless the following box is checked:

☒ a été déposée le 06 juin 2000 sous le numéro de demande des Etats-Unis ou le numéro de demande international PCT PCT/FR 00/01546 et modifiée le / (le cas échéant).

☐ was filed on _____ as United States Application Number or PCT International Application Number _____ and was amended on _____ (if applicable).

Je déclare par le présent acte avoir passé en revue et compris le contenu de la description ci-dessus, revendications comprises, telles que modifiées par toute modification dont il aura été fait référence ci-dessus.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

Je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

French Language Declaration

Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, § 119(a)-(d) ou § 365(b) du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur ou, en vertu du Titre 35, § 365(a) du même Code, sur toute demande internationale PCT désignant au moins un pays autre que les Etats-Unis et figurant ci-dessous et, en cochant la case, j'ai aussi indiqué ci-dessous toute demande étrangère de brevet, tout certificat d'inventeur ou toute demande internationale PCT ayant une date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365 (b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior foreign applications Demande(s) de brevet antérieure(s) dans un autre pays:			Priority claimed Droit de priorité revendiqué	
(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)		
99.07337	FRANCE	10 June 1999	[X] Yes Oui	[ ] No Non
			[ ] Yes Oui	[ ] No Non
			[ ] Yes Oui	[ ] No Non

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 119(e) du Code des Etats-Unis, de toute demande de brevet provisoire effectuée aux Etats-Unis et figurant ci-dessous.

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)
(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 120 du Code des Etats-Unis, de toute demande de brevet effectuée aux Etats-Unis, ou en vertu du Titre 35, § 365(c) du même Code, de toute demande internationale PCT désignant les Etats-Unis et figurant ci-dessous et, dans la mesure où l'objet de chacune des revendications de cette demande de brevet n'est pas divulgué dans la demande antérieure américaine ou internationale PCT, en vertu des dispositions du premier paragraphe du Titre 35, § 112 du Code des Etats-Unis, je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations, dont j'ai pu disposer entre la date de dépôt de la demande antérieure et la date de dépôt de la demande nationale ou internationale PCT de la présente demande:

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)	(Status) (patented, pending, abandoned) (Statut) (breveté, en cours d'examen, abandonné)
(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)	(Status) (patented, pending, abandoned) (Statut) (breveté, en cours d'examen, abandonné)

Je déclare par le présent acte que toute déclaration ci-incluse est, à ma connaissance, véridique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour véridique; et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la Section 1001 du Titre 18 du Code des Etats-Unis, et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

French Language Declaration

POUVOIRS: En tant que l'inventeur cité, je désigne par la présente l'(les) avocat(s) et/ou agent(s) suivant(s) pour qu'ils poursuive(nt) la procédure de cette demande de brevet et traite(nt) toute affaire s'y rapportant avec l'Office des brevets et des marques: *(mentionner le nom et le numéro d'enregistrement).*

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*

(7)

ROBERT J. PATCH, Reg No. 17,355
ANDREW J. PATCH, Reg. No. 32,925
ROBERT F. HARGEST, Reg. No. 25,590
BENOÎT CASTEL, Reg. No. 35,041
ERIC JENSEN, Reg. No. 37,855
THOMAS W. PERKINS, Reg. No. 33,027
ROLAND E. LONG, JR., Reg. No. 41,949

Adresser toute correspondance à:

Send Correspondence to:

YOUNG & THOMPSON
SECOND FLOOR
745 SOUTH 23RD STREET
ARLINGTON, VA 22202

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(name and telephone number)

ROBERT J. PATCH, 703/521-2297

Nom complet du seul ou premier inventeur Jean-Pierre GRASA	Full name of sole or first inventor
Signature de l'inventeur Date 13/10/2001	Inventor's signature Date
Domicile Saint Julien de Peyrolas, France	Residence
Nationalité Française	Citizenship
Adresse Postale Planjole, 30760 SAINT JULIEN DE	Post Office Address
PEYROLAS (FRANCE)	
Nom complet du second co-inventeur, le cas echeant	Full name of second joint inventor, if any
Signature de l'inventeur Date	Second inventor's signature Date
Domicile	Residence
Nationalité	Citizenship
Adresse Postale	Post Office Address

(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)